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THE ESTIMATION OF SUGARS IN CONDENSED MILK.[1]

By W. D. Bigelow and K. P. McElroy.

Abstract read before the Washington Chemical Society, February 9, 1893.

IN the course of the work with canned goods, which has been carried on in this laboratory during the past year, it was desired to examine condensed milk.

The brands of condensed milk are commonly divided into two classes,—those which contain cane sugar, and those which do not. When cane sugar is absent, milk sugar may be determined by any of the methods which are used for its estimation in fresh milk. In the analysis of condensed milk which contains cane sugar, it has usually been taken for granted that invert sugar was absent. Dr. Wiley, by whose direction this work was undertaken, desired a method for the estimation of the sugars present in condensed milk, which would take into consideration the possible presence of invert sugar. It was at his instance, therefore, that the work which forms the basis of this paper was undertaken. It is not our intention at this time to review all the methods which have been suggested in the analysis of condensed milk, but merely to record some ideas which have suggested themselves and some methods which have been developed in the progress of this work.

Among the methods which have been suggested for the determination of cane sugar in the presence of milk sugar the following may be cited as being the most important:

(1) In the method which seems to be most frequently employed, the total solids are estimated by evaporating a portion of the liquid and drying it to constant weight; casein, fat, and ash are estimated by the ordinary methods; the milk sugar by reduction with an alkaline copper solution; and the amount of cane sugar is obtained by deducting the milk solids from the total solids. Aside from the fact that a "difference" method is never satisfactory, this method is open to the objection that whatever invert sugar may be present is estimated as milk sugar, and the presence of invert sugar is always possible where

[1] Read before the World's Congress of Chemists, Chicago, August 22, 1893.

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cane sugar has been used in preserving condensed milk. It is certainly advisable to employ a method which will detect it when present.

(2) The method suggested by Muter[1] is well worthy of attention. In this method, ten grams[2] of the milk are poured on four grams of hydrated calcium sulphate in a basin and evaporated to perfect dryness with frequent stirring so that nothing sticks to the basin. The dry residue is powdered, macerated with ether, thrown on a dry filter over a tared beaker, and percolated with ether until free from fat. The ether is then evaporated off, and the beaker plus fat weighed, from which the percentage of fat is ascertained. The filter and contents are then transferred to a beaker, twenty cc. of hot (not boiling) water added, and the whole well stirred. Thirty cc. of alcohol (sixty per cent.) are then added, and the mixture is allowed to cool with occasional stirring. When cool it is thrown on a filter placed over a long graduated measure and washed with proof spirits (two volumes of water to one of sixty per cent. alcohol) until the filtrate measures 120 cc., at which point the extraction is usually complete. The filtrate is then divided into two equal parts and one portion evaporated in a platinum dish, and then placed in a drying oven at 100° till constant weight is obtained. The weight is then noted, the dish ignited for some time at a dull red heat and again weighed, when the total weight less dish plus ash gives the total sugar, which is multiplied by twenty to get percentage. Evaporate the alcohol from the other portion of the filtrate, wash into a beaker, and determine lactose by means of Fehling's solution. The weight of cuprous oxide thus obtained is multiplied by twenty and calculated from 146 to 100. The amount of milk sugar thus found is deducted from the total sugar, and the result, if over 0.5, is put down as cane sugar. If over 0.5 and under 1.0, a rebate of 0.2 per cent., and if over 1.0 and under 1.5, an allowance of 0.1 per cent., is to be made. But if the amount reaches over 2.0 per

[1] *Analyst, 1880*, 5, 37.

[2] This method is proposed for the examination of fresh milk which has been adulterated with condensed milk. If it is desired to apply it to condensed milk, two to three grams should be taken instead of ten grams, and the corresponding correction made in the method of calculation.

cent. it may be taken as it stands. This method is somewhat tedious, and in general is open to the same objections as the first one cited. It is also found that when a large amount of cane sugar is present considerable difficulty is experienced in the extraction of both sugar and fat.

(3) The inversion of the cane sugar by means of mineral acids: A number of methods have been suggested which may be classed under this head. They are all open to the objection that milk sugar also undergoes hydrolysis when subjected to the action of mineral acids. It has been suggested that a blank containing the same amount of milk sugar as the milk under examination be run with each experiment. This is not practicable, however, as the action of milk sugar under these circumstances is far from uniform.

(4) Shenstone[1] determines both sugars by the aid of the polariscope, milk sugar by copper reduction, and estimates the cane sugar by difference. His method, briefly stated, is as follows: Dilute thirty grams of condensed milk, boil to obtain a normal rotation of the milk sugar, cool, make up to ninety-seven cc. with water, and add three cc. of acid mercurous nitrate. The solution is then mixed by pouring between two beakers, and filtered. One portion of the filtrate is then polarized; this gives the combined readings of sucrose and lactose. Ten cc. of the filtrate are then diluted to 100 cc., and the milk sugar is determined by either Fehling's or Pavy's solution; the combined reading obtained from both sugars less that representing the amount of milk sugar present being equal to the polarization of the cane sugar present. This method has also the disadvantage that it does not indicate the presence of invert sugar. Another objection which may be urged is that inversion is likely to be caused by the nitric acid present in the solution. The author states that if the sugars be determined as soon as possible after the clarification of the milk no inversion can be detected. It is evident, however, that should even a slight inversion occur, a decided error in the amount of sucrose obtained would result. In the first place, the invert sugar would reduce sufficient cuprous oxide to indicate almost

[1] *Analyst*, 1888, 13, 222.

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twice its weight of milk sugar. Then, of course, the polarization of the combined sugars would be reduced. Thus, the sucrose would be obtained by subtracting a figure too large for the amount of milk sugar from one too small for the combined polarization of the two sugars.

(5) Stokes and Bodmer[1] have suggested that the cane sugar be determined by inversion with citric acid. The details of their method are as follows: Dilute the milk and place in a burette. Hang on to the lower end of the burette a flask (100 cc.) by means of a rubber tube fitted with a screw clip. The flask should be provided with another tube for the exit of ammonia vapors. Place in the flask forty cc. of Pavy's solution, boil, and gradually add the sugar solution till the blue color disappears. Read the burette. Into another flask containing forty cc. of Pavy's solution run the amount of sugar solution (less two-tenths) found necessary to decolorize in the first experiment, and boil. Should the liquid remain blue, it shows that the first reading was correct within 0.1 cc. Next boil another portion of the diluted milk with citric acid in sufficient amount to form two per cent. of the solution, for ten minutes, cool, neutralize with ammonia and repeat the titration. Cane sugar is completely inverted by boiling with two per cent. of citric acid, while milk sugar is not affected. The reducing power of milk sugar may be taken as being fifty-two per cent. of that of glucose, using Pavy's solution. The dilution of the milk should be such that from six to twelve cc. are required for the titration. This method does not seem to have come into general use, although it has been tried by several and is said to give good results. The invert sugar is not taken into account. Shenstone[2] proposes a modification of the apparatus used by Stokes and Bodmer by which he replaces the ammonia driven off during the operation.

The Determination of Cane Sugar.—It is well known that in the inversion of cane sugar by means of acids, the results are greatly influenced by the apparatus used and the details of the operation. For this reason it was thought best to examine the

[1] *Analyst, 1885*, **10**, 62.

[2] *Analyst, 1888*, **13**, 222.

methods for the inversion of cane sugar by means of invertase.[1] This method was first proposed by Kjeldahl[2] for the estimation of the sucrose in barley and malt. He found that results obtained by its use were more accurate than those obtained by inversion with acids. Later it was used by O'Sullivan[3] for the estimation of sucrose in cereals. In 1890, O'Sullivan and Tompson[4] made a careful study of this method and published an account of their work together with a bibliography of invertase and directions for its preparation.

Instead of preparing the invertase itself as they here directed, we followed the method suggested by them in a later paper[5] and used the yeast liquor obtained by the decomposition of brewers' yeast.

For the preparation of this liquid, brewers' yeast was allowed to stand one month at the temperature of 15°. The product, which was then mostly liquid, was filtered, and for the purpose of preservation alcohol was added till the solution contained ten to twelve per cent. absolute alcohol. The mixture was then allowed to stand for a few days and filtered.

The first series of experiments was made to determine the amount of yeast liquor required to obtain complete inversion. The amount of yeast liquor used varied from one to three cc. and the time was four hours.

About 200 grams of granulated sugar were dissolved in one liter of water. For each inversion fifty cc. of this solution were transferred to a hundred cc. flask. Two of these portions were made up to the 100 cc. mark, and the solutions polarized without inversion to obtain the direct reading. The solution polarized 38.3° on the Schmidt and Haensch half-shadow instrument in a 200 mm. tube. The flasks which contained sugar intended for inversion were placed in a large water bath, the temperature of which was held at 55° throughout the operation. As

[1] Richmond and Boseley (*Analyst, July, 1893*) suggest that this method would probably be suitable for the determination of cane sugar in condensed milk. Our own work, however, was done before that time.

[2] *Meddelelser, 1881*, 337.

[3] *J. Chem. Soc., Trans., 1886*, **49**, 58.

[4] *J. Chem. Soc., Trans., 1890*, **57**, 834.

[5] *J. Chem. Soc., Trans., 1891*, **59**, 46.

soon as the proper temperature was reached the yeast liquor was added. In order to hold the flasks in position in the bath, a frame was constructed of parallel strips placed about one inch apart and small nails were driven at intervals of about two inches along the top of the strips. The necks of the flasks were then placed between these strips and held in position by means of rubber bands which were stretched across between the nails. At the end of four hours the operation was stopped. A little corrosive sublimate was added to the contents of each flask to prevent any fermentation which might be caused by the yeast as the solution cooled. Some alumina cream was also added to assist in the clarification. The solutions were then cooled to about the temperature of the room, made up to the 100 cc. mark, thoroughly mixed by shaking, filtered, and polarized. The result of this experiment is given in table 1.

TABLE 1.

Yeast liquor, cc.	Total liquid in cc.	Cane sugar added, grams.	Cane sugar found, grams.
1	51	9.976	9.690
1	51	9.976	9.664
2	52	9.976	9.846
2	52	9.976	9.820
3	53	9.976	9.898
3	53	9.976	9.768
3	53	9.976	9.716

On examining this table, it will be evident that the amount of cane sugar indicated is too low in every case.

Another series of inversions was then started in which the sucrose solution was of the same strength as in the previous series, though only half as much (about five grams) was taken for each inversion. The direct polarization of one of these portions was found to be 19.15° on the sugar scale. The time allowed was five hours and the amount of yeast liquor used was five cc. The results of this series will be found in table 2, which is given below:

TABLE 2.

Yeast liquor, cc.	Total liquid in cc.	Cane sugar added, grams.	Cane sugar found, grams.
5	30	4.988	5.001
5	30	4.988	4.975
5	30	4.988	4.975
5	30	4.988	5.001
5	30	4.988	4.988
7	32	4.988	5.001

It was also suggested by O'Sullivan and Tompson[1] that brewers' yeast be used instead of invertase or yeast liquor. They directed that fresh brewers' yeast be added to the solution to be inverted to the amount of about one-tenth the weight of the sucrose present. On account of the greater uniformity of ordinary compressed yeast, however, and the ease with which it can always be obtained, a series of inversions was made with it and its action compared with that of the yeast liquor used in the preceding experiments. For this purpose the cane sugar solution was prepared as before, and some portions containing ten, and others containing five grams, were taken for the inversion. The time allowed for this series was four hours. The results obtained by this series of inversions are given in table 3.

TABLE 3.

Yeast, grams.	Total liquid in cc.	Cane sugar added, grams.	Cane sugar found, grams.
1	60	9.976	9.989
1	60	9.976	9.872
1	60	9.976	9.872
2	70	9.976	9.846
2	70	....	....
2	70	....	....
2	45	4.988	4.845
2	45	4.988	4.897

These results are too low. In order to determine whether this is due to the fact that not enough time was allowed for complete inversion, or an insufficient amount of yeast was employed, two more sets of inversions were begun, in one of which (table 4), five grams, in the other (table 5), ten grams, of cane sugar were employed.

TABLE 4.

Yeast, grams.	Total liquid in cc.	Cane sugar added, grams.	Cane sugar found, grams.
1	25	4.988	4.975
1	25	4.988	4.988
1	25	4.988	4.949
2	25	4.988	4.975
2	25	4.988	4.949
2	25	4.988	5.001

[1] *J. Chem. Soc., Trans., 1891*, 59, 46.

TABLE 5.

Yeast, grams.	Total liquid in cc.	Cane sugar added, grams.	Cane sugar found, grams.
2	50	9.976	9.950
2	50	9.976	9.950
5	50	9.976	9.976
5	50	9.976	10.002
5	50	9.976	9.976
5	50	9.976	9.976

In each case the inversion was allowed to proceed for five hours. Several of the inversions were allowed to proceed for seven hours, and in no case was any destructive action upon the invert sugar evident.

From these results we infer:

(1) That the results obtained by means of compressed yeast compare very favorably with those obtained by the use of yeast liquor, and since this is more readily obtained and is more convenient it is to be preferred.

(2) That the time necessary for complete inversion is five hours.

(3) That while fair results may be obtained with an amount of yeast equal to one-fifth the weight of the sucrose present, the best results are to be obtained with an amount equal to one-half the weight of the sucrose it is desired to invert. It will be remembered that O'Sullivan and Tompson[1] added brewers' yeast to the amount of one-tenth the weight of sucrose which it was desired to invert. This is due to the fact that the per cent. of invertase contained in compressed yeast is less than in brewers' yeast.

The next step was to determine the effect of invertase upon a pure solution of milk sugar. There seems to be no literature upon this subject. Dastre[2] makes the statement that lactose is inverted by the invertive ferment present in yeast. He seems, however, to base his opinion on the fact that lactose disappears in the presence of yeast, and cites no experiment which would seem to justify the statement that this sugar is hydrolyzed.

[1] *J. Chem. Soc., Trans., 1891*, 59, 46.

[2] *Compt. Rend., 1883*, 96, 932.

Milk sugar was dissolved in water, the solution boiled to obtain the normal rotation of milk sugar, cooled to room temperature, and portions of fifty cc. each measured into 100 cc. flasks. Two of these were diluted to the mark and polarized. The reading was 5.2°. The others were subjected to the action of invertase for five hours at 55°, some with yeast liquor, others with compressed yeast. The polarization of the solution before and after the operation is given in tables 6 and 7.

TABLE 6.

Yeast liquor, cc.	Total liquid in cc.	Original polarization.	Polarization after inversion.
1	51	5.2	5.3
1	51	5.2	5.1
2	52	5.2	5.1
2	52	5.2	5.2
2	52	5.2	5.2
3	53	5.2	5.3
3	53	5.2	5.2

TABLE 7.

Yeast, grams.	Total liquid in cc.	Original polarization.	Polarization after inversion.
2	50	5.2	5.1
2	50	5.2	5.3
2	50	5.2	5.3
5	50	5.2	5.2
5	50	5.2	5.0

From these figures we feel justified in saying that the inversion noted by Dastre must have been due to some other cause than the influence of the invertase present in yeast.

The fact being thus established that milk sugar is not affected by the action of invertase, it was next undertaken to invert cane sugar in the presence of milk sugar by this method.

Solutions of the two sugars were prepared of about the same strength as was used in the preceding experiments. About 600 grams of granulated sugar were dissolved in three liters of water. Approximately 100 grams of milk sugar were dissolved in three liters of water. This solution was boiled for some time, and then cooled to room temperature. Fifty cc. of the cane sugar solution diluted to 100 cc. and polarized gave a reading of 38.2° on the sugar scale.

In this set of inversions, as in the previous ones, 100 cc.

flasks were used. Into each flask were placed fifty cc. of the sucrose solution and twenty-five cc. of the lactose solution, making about twelve parts of the former to one of the latter. The operation was conducted just as in the inversions of the cane sugar alone, and was allowed to proceed for five hours. The results are given in table 8.

TABLE 8.

Yeast liquor, cc.	Milk sugar, grams.	Total liquid in cc.	Cane sugar added, grams.	Cane sugar found, grams.
1	0.86	76	9.950	9.794
2	0.86	77	9.950	9.898
2	0.86	77	9.950	9.924
3	0.86	77	9.950	9.950
3	0.86	78	9.950	9.924
3	0.86	78	9.950	9.950

In this experiment the amount of yeast liquor used varied from one to three cc. for each inversion. It will be seen from the table that the inversion was not complete in those samples which contained less than three cc. of yeast liquor.

It was then attempted to invert cane sugar mixed with about one-third its weight of milk sugar. For that purpose twenty-five cc. of a cane sugar solution, fifty cc. of a milk sugar solution, of the same strength as was used in the preceding experiments, were transferred to each flask used in this series of inversions. Twenty-five cc. of the cane sugar diluted to 100 cc. and polarized gave a reading of 19.1° and contained about five grams of sucrose. After five hours the inversion was interrupted. The results are given in table 9.

TABLE 9.

Yeast liquor, cc.	Total liquid in cc.	Milk sugar added, grams.	Cane sugar added, grams.	Cane sugar found, grams.
1	76	1.716	4.975	4.923
1	76	1.716	4.975	4.975
1	76	1.716	4.975	4.975
2	77	1.716	4.975	4.923
2	77	1.716	4.975	4.949
2	77	1.716	4.975	4.975
3	78	1.716	4.975	4.975
3	78	1.716	4.975	4.949
3	78	1.716	4.975	4.975
3	78	1.716	4.975	4.949

It was next desired to study the influence of compressed yeast on solutions containing both sucrose and lactose in the proportion of twelve of the former to one of the latter. Each portion contained about ten grams of cane sugar. The inversion was interrupted after four hours. In table 10 are given the results of the inversion.

TABLE 10.

Yeast, grams.	Total liquid in cc.	Milk sugar added, grams.	Cane sugar added, grams.	Cane sugar found, grams.
1	85	0.858	9.950	9.820
1	85	0.858	9.950	9.898
1	85	0.858	9.950	9.924
1	85	0.858	9.950	9.820
1	85	0.858	9.950	9.898
	95	0.858	9.950	9.872
2	95	0.858	9.950	9.898

These results are rather low, probably on account of the fact that sufficient time was not allowed for the complete inversion of this amount of sucrose.

The same amount of cane sugar was then inverted in the presence of twice the amount of milk sugar. The time allowed was in some cases four (table 11), in some, five hours (table 12).

TABLE 11.

Yeast, grams.	Total liquid in cc.	Milk sugar added, grams.	Cane sugar added, grams.	Cane sugar found, grams.
1	100	1.716	9.950	9.898
1	100	1.716	6.950	9.846
1	100	1.716	9.950	9.950
2	100	1.716	9.950	9.794
2	100	1.716	9.950	9.846
2	100	1.716	9.950	9.846

TABLE 12.

Yeast, grams.	Total liquid in cc.	Milk sugar added, grams.	Cane sugar added, grams.	Cane sugar found, grams.
2	100	1.716	9.950	9.898
2	100	1.716	9.950	9.976
2	100	1.716	9.950	9.950
5	100	1.716	9.950	9.924
5	100	1.716	9.950	9.950

The examination of these tables confirms the former experi-

ments with regard to the time necessary for complete fermentation.

In this case, as in all others in which the volume of the liquid was too great to make use of a 100 cc. flask, a 200 cc. flask was used instead, and the reading obtained was multiplied by two.

A mixture of cane and milk sugar was then made, in which the sugars were present in about the same proportion as they are usually found in condensed milk, *i. e.*, three parts of cane sugar to one of milk sugar. Approximately five grams of sucrose were present. The time allowed for this set of inversions was five hours, and the result, as will be seen in table 13, was entirely satisfactory.

TABLE 13.

Yeast, grams.	Total liquid in cc.	Milk sugar added, grams.	Cane sugar added, grams.	Cane sugar found, grams.
1	75	1.716	4.975	4.983
1	75	1.716	4.975	4.949
1	75	1.716	4.975	4.975
1	75	1.716	4.975	4.975
1	75	1.716	4.975	4.923
2	75	1.716	4.975	5.001
2	75	1.716	4.975	4.949
2	75	1.716	4.975	4.975

A sample of whole milk was then taken, fifty cc. introduced into a 100 cc. flask, three cc. mercuric iodide solution[1] and a little alumina cream added, the solution made up to the mark, filtered, and polarized. Similar portions of the milk were heated to 80° to destroy any ferments which might be present, allowed to cool to 55° and treated as before; some with yeast liquor and others with compressed yeast. Some of these were kept at this temperature for four hours, and others for five hours. Then they were clarified as before, cooled, made up to the mark, filtered, and polarized. On examining table 14, it will be seen that, allowing for the usual error of polarization, the milk sugar had not been in any way affected by the action of the invertase. A portion of this milk was diluted to some extent before the portions for inversion were measured out. These, as will be seen by the table, read 6.1° on the sugar scale before inversion.

[1] Potassium iodide 33.2 grams, mercuric chloride 13.5 grams, acetic acid 20 cc., water 64 cc.

TABLE 14.

Yeast liquor, cc.	Total liquid in cc.	Milk, cc.	Polarization before inversion.	Polarization after inversion.	Time in hours.
3	53	50	8.2	8.1	4
3	53	50	8.2	8.2	4
3	53	50	8.2	8.3	4
3	53	50	8.2	8.2	4
3	53	50	8.2	8.2	4
3	53	50	8.2	8.2	4
3	53	50	8.2	8.1	4
3	53	50	8.2	8.2	4
3	53	50	8.2	8.3	4
3	53	50	8.2	8.3	4
3	53	50	8.2	8.2	5
3	53	50	8.2	8.2	5
3	53	50	8.2	8.2	5
3	53	50	8.2	8.1	5
3	53	50	6.1	6.1	5
3	53	50	6.1	6.0	5
5	55	50	6.1	5.9	5
5	55	50	6.1	6.1	5
5	55	50	6.1	6.1	5
5	55	50	6.1	6.2	5

The influence of compressed yeast upon whole milk was next tried. This series is a repetition of the previous one, with the substitution of compressed yeast for yeast liquor. The results are given in table 15.

TABLE 15.

Yeast, grams.	Total liquid in cc.	Milk, cc.	Polarization before inversion.	Polarization after inversion.	Time in hours.
2	70	50	8.2	8.0	4
2	70	50	8.2	8.2	4
2	70	50	8.2	8.2	4
2	70	50	8.2	8.3	4
2	70	50	8.2	8.0	4
2	70	50	8.2	8.2	4
2	70	50	8.2	8.2	4
2	70	50	8.2	8.2	4
2	70	50	8.2	8.1	4
2	70	50	8.2	8.2	5
2	70	50	8.2	8.3	5
2	70	50	6.1	6.0	5
2	70	50	6.1	6.1	5
2	70	50	6.1	6.1	5

From these results it appears that if there is any inversion of milk sugar produced by the action of invertase, the amount must be so small that it can not be detected by the polariscope.

The next step taken was to mix a solution of cane sugar with whole milk, and invert the sucrose in this mixture. For this purpose approximately 400 grams of granulated sugar were dissolved in one liter of water. Twenty-five cc. of this solution diluted to 100 cc. gave a reading of 39.0° on the sugar scale. Fifty cc. of the milk which was to be used in this experiment were placed in a 100 cc. flask, clarified as given above by means of mercuric iodide dissolved in acetic acid, and alumina cream, made up to 100 cc., filtered, and polarized. The reading was 8.2°. To another fifty cc. of the same milk twenty-five cc. of the cane sugar solution were added, clarified as before, made up to the mark, filtered, and polarized. The filtrate read 48.2°. From these figures the volume of the solids precipitated from fifty cc. of the milk was estimated to be 2.5 cc. For the purpose of inverting cane sugar in solution in milk, portions of fifty cc. each of the milk were measured into 100 cc. flasks, twenty-five cc. of the solution of cane sugar were added to each portion, and the mixture treated with yeast liquor in the same manner as in the inversion of sucrose in aqueous solution. As soon as the contents of the flasks had reached the temperature of 55°, the yeast liquor was added. This temperature was held constant for four hours. Three cc. of the solution of mercuric iodide in acetic acid and a little alumina cream were then added, the flasks shaken gently, cooled, made up to the mark, filtered, and the filtrate polarized. The results obtained by this method are given in table 16.

TABLE 16.

Yeast liquor, cc.	Total liquid in cc.	Milk, cc.	Cane sugar added, grams.	Cane sugar found, grams.
3	80	50	10.158	10.054
3	80	50	10.158	10.080
3	80	50	10.158	10.132
3	80	50	10.158	10.132
3	80	50	10.158	9.976

A solution of cane sugar was also made which contained half the amount of sucrose given above. Similar mixtures inverted

as in the preceding experiment for four hours showed that the sucrose was completely inverted, as may be seen in table 17.

TABLE 17.

Yeast liquor, cc.	Total liquid in cc.	Milk, cc.	Cane sugar added, grams.	Cane sugar found, grams.
3	80	50	5.079	5.079
3	80	50	5.079	5.079
5	80	50	5.079	5.105

A comparison of tables 16 and 17 shows that better results were obtained with five grams of sucrose than with ten grams. It was thought that a five hour inversion would give better results in the presence of ten grams of sucrose. Twenty-five cc. of an aqueous solution containing that amount of cane sugar were added to fifty cc. of milk, and the mixture subjected to the action of yeast liquor for five hours. The results as given in table 18 would seem to indicate that five hours are necessary for complete inversion.

TABLE 18.

Yeast liquor, cc.	Total liquid in cc.	Milk, cc.	Cane sugar added, grams.	Cane sugar found, grams.
3	80	50	10.158	10.162
3	80	50	10.158	10.158
3	80	50	10.158	10.210
3	80	50	10.158	10.132
3	80	50	10.158	10.162
3	80	50	10.158	10.106
3	80	50	10.158	10.210
3	80	50	10.158	10.210
3	80	50	10.158	10.236
4	80	50	10.158	10.158
4	80	50	10.158	10.184
4	80	50	10.158	10.158
4	80	50	10.158	10.158
4	80	50	10.158	10.080
4	80	50	10.158	10.236

It was next desired to study the influence of compressed yeast on a solution of sucrose in milk. For this purpose about 180 grams of granulated sugar were dissolved in one liter of water and the solution polarized. Twenty-five cc. of this solution were mixed with fifty cc. of milk for each inversion. As in all former cases compressed yeast gave the same result as the

liquor prepared from brewers' yeast had done. The results are given in table 19.

TABLE 19.

Yeast, grams.	Total liquid in cc.	Milk, cc.	Cane sugar added, grams.	Cane sugar found, grams.
2	75	50	4.611	4.637
2	75	50	4.611	4.585
2	75	50	4.611	4.637
2	75	50	4.611	4.611
2	75	50	4.611	4.585
2	75	50	4.611	4.611

The time allowed for this operation was but four hours, but the amount of sugar in each flask was less than five grams and the inversion was complete.

It was next undertaken to invert ten grams of cane sugar by means of yeast. This amount of granulated sugar dissolved in twenty-five cc. of water was added to fifty cc. of milk, the mixture clarified, made up to the mark, filtered, and the filtrate polarized as before. The reading was 48.2°. Fifty cc. of the milk treated in the same way gave a reading of 8.2°. The reading of the sucrose added, therefore, was taken as 40.0°. At the end of four hours the operation was interrupted. The sucrose found is given in table 20.

TABLE 20.

Yeast, grams.	Total liquid in cc.	Milk, cc.	Cane sugar added, grams.	Cane sugar found, grams.
2	75	50	10.158	10.132
2	75	50	10.158	10.080
2	75	50	10.158	10.210
2	75	50	10.158	10.158
2	75	50	10.158	10.132
2	75	50	10.158	10.206
2	75	50	10.158	10.132

The time in this case was not sufficient to permit complete inversion.

Other portions of the solutions used in the preceding set of inversions were treated in the same manner, except that the inversion was allowed to proceed for five hours. The results are given in table 21.

TABLE 21.

Yeast, grams.	Total liquid in cc.	Milk, cc.	Cane sugar added, grams.	Cane sugar found, grams.
2	75	50	10.158	10.184
2	75	50	10.158	10.184
2	75	50	10.158	10.184
2	75	50	10.158	10.210
2	75	50	10.158	10.210
2	75	50	10.158	10.158
2	75	50	10.158	10.028
2	75	50	10.158	10.158
2	75	50	10.158	10.210

The comparison of tables 19, 20, and 21, confirms the conclusions drawn from the results of the corresponding work with the liquor from brewers' yeast.

From these experiments it is evident that this method may be applied to the determination of sucrose in the presence of milk with results which are entirely satisfactory.

Four determinations of sucrose in one of our most popular brands of condensed milk gave the following results:

40.0 per cent. 40.0 per cent. 39.9 per cent. 40.2 per cent.

After this article was prepared Richmond and Boseley[1] made the statement that the polariscope could not be used for the examination of condensed milk, because of the effect of the heat necessary to condense the milk on the optical properties of milk sugar. Thus far we have met with no difficulty from this source.

Milk Sugar.—The method for the determination of milk sugar which is suggested below is intended to be used only in the presence of invert sugar. It is evident that when this is present the milk sugar can be determined neither by copper reduction nor by polarization.

Under these circumstances the most convenient solution of the problem seemed to be the destruction of the cane and invert sugars by fermentation, and the subsequent determination of the lactose by means of the polariscope, or by the copper reduction method. It is, however, well known that the complete fermentation of sucrose is a difficult thing to accomplish, the trouble being largely due to the lactic ferment which invariably occurs in saccharine liquids fermenting under ordinary circumstances.

[1] *Analyst*, *1893*, **18**, 141.

It is equally well known that lactose disappears in the presence of common yeast. This is also probably due to the lactic ferment. Certain inorganic acids and salts, especially hydrofluoric acid and fluorides, have the power of preventing the lactic acid fermentation, and of accelerating, retarding, or even entirely stopping the yeast fermentation, according to the amount employed.

Although fluorides have been extensively used for this purpose in breweries and distilleries, no attempt has yet been made to apply their action to an analytical method.

It seemed desirable then to investigate this subject in order to determine whether their action was sufficiently complete to form the basis of an analytical method. Our first experiments were made to determine whether cane sugar could be completely fermented by yeast in the presence of fluorides. About 1,200 grams of granulated sugar were dissolved in water and the solution made up to six liters. One hundred and seventeen portions of this solution of fifty cc. each (each containing about ten grams of sucrose) were placed in flasks with one-half cake Fleischman's compressed yeast, together with amounts of potassium fluoride varying from five to thirty-five mgms. to 100 cc. of the solution.

These flasks were divided into three lots which were designated as lots A, B, and C. Lot A was kept at a temperature of from 11° to 15°, lot B from 20° to 25°, and lot C in a room the temperature of which varied from 15° to 35°.

In each of these lots the flasks were arranged in thirteen sets of three each. The flasks of each set all contained the same amount of fluoride, and a blank containing no sugar was run with each set. One flask from each set was used for qualitative tests with Fehling's solution. The others were polarized.

After standing three days all samples showed heavy copper reduction. After five days all samples of lot A showed heavy copper reduction, while those samples of lots B and C, which contained the lower amounts of fluorides, showed but light copper reduction. Lot C had progressed farther than lot B.

After seven days the fermentation in lots B and C was almost complete. The fermentation in lot B seemed to be the more

favorable. After eight days the Fehling's solution gave but a slight reaction in lot B and but slightly heavier in lot C. On the tenth day lots B and C were polarized and the sugar was found to be entirely destroyed. Lot A required a much longer time for complete fermentation than lots B and C, sugar still being present after three weeks' standing. After twenty-five days, however, the fermentation was complete. From these experiments we conclude:

(1) That it is possible and entirely practicable to completely ferment cane sugar by means of yeast in the presence of potassium fluoride.

(2) That with from fifteen to thirty mgms. of fluoride per 100 cc. of the solution the fermentation is most satisfactory.

(3) That the best temperature for complete fermentation is from 20° to 25°; and

(4) That under these circumstances eight days is sufficient for complete fermentation.

The next step was to determine the action of yeast on milk sugar in the presence of fluorides. Approximately 160 grams of milk sugar were dissolved in one liter of water and the solution boiled for some minutes. One hundred cc. of this solution placed in a 200 cc. flask, filled up to the mark and polarized, gave a reading of 24.4° on the sugar scale. Similar portions of the solution were treated with yeast and fluoride as shown in the table and set aside for a number of days. In this series, however, there were six determinations in each set instead of three as in the preceding set. Beginning with the eighth day these solutions were polarized at intervals of from two to three days. In table 22 are given all the readings which were taken in this series.

TABLE 22.

Lactose, grams.	Mgms. KF, per 100 cc.	Total liquid in cc.	Original polarization.	Polarization after 8 days.	Polarization after 11 days.	Polarization after 13 days.	Polarization after 16 days.	Polarization after 18 days.	Polarization after 35 days.
8	..	100	24.4	23.4	22.8	21.6	20.4	....	....
8	15	103	24.4	24.4	24.4	24.0	23.6	....	....
8	20	105	24.4	24.4	24.4	24.2	24.0	....	....
8	30	106	24.4	24.4	24.4	24.3	23.8	....	20.0
8	45	110	24.4	24.4	24.4	24.3	24.0	....	18.6
8	60	112	24.4	24.6	24.4	24.2	24.3	24.2	19.2

As before said, it is usually stated that milk sugar is slowly consumed by common yeast. Our own experiments without the use of fluorides confirm this idea, as is shown in table 22. It will be seen that on the eleventh day the sample which contained no fluoride read 1.6° lower than those which contained fluoride. It was also impossible to obtain a good clarification on that day, probably on account of the lactic acid ferment which was growing in numbers and which passes through the filter and renders the filtrate turbid. On the sixteenth day the reading was made only with extreme difficulty, while on the eighteenth day it was impossible to effect a clarification which would enable us to read the solution at all.

The next step was the fermentation of mixtures of cane and milk sugar. Separate solutions of both sugars were made in the same manner as in the two preceding series of experiments. This series was treated just as the previous one except that six grams of sucrose were introduced into each flask with the eight grams of lactose. At the temperature at which these experiments were conducted (18° to 20°) thirteen days were required for the complete fermentation of the sucrose. It is believed that ten days would have been sufficient if the temperature had been kept at about 25°. The milk sugar solution used polarized 24.3°. All the readings taken in this series are given in table 23.

TABLE 23.

Mgms. KF. per 100 cc.	Total liquid in cc.	Polarization after 5 days.	Polarization after 8 days.	Polarization after 11 days.	Polarization after 13 days.	Polarization after 16 days.	Polarization after 18 days.	Polarization after 35 days.
..	150	23.1	24.0	23.8	23.8	24.0	....	....
15	154	24.1	23.7	24.0	24.0	24.1	24.0	23.4
20	156	23.4	23.1	24.0	24.3	24.3	24.3	23.1
30	158	23.4	23.4	23.7	24.3	24.3	24.3	23.1
40	162	23.1	23.1	24.0	24.3	24.4	24.3	23.7
55	166	23.1	23.7	24.0	23.8	24.0	24.3	24.3

On examining this table it will be seen that the sample which contained no fluoride, and the one which contained but fifteen mgms., did not reach the polarization of the lactose, for before the fermentation of the sucrose was complete the lactic acid fermentation set in to such an extent as to retard the action of the yeast. On the eleventh day the filtrate from the solution con-

taining no fluoride was slightly turbid; on the thirteenth day the reading could be taken only with difficulty, while on the sixteenth day only an approximate reading could be obtained. On the thirteenth day the filtrate from the solution containing fifteen mgms. of fluoride was slightly turbid, and this turbidity increased with each successive polarization of samples containing this amount of fluoride.

A mixture of ten grams of cane sugar and eight grams of milk sugar was next subjected to the action of yeast in the presence of potassium fluoride. The original polarization of the milk sugar was 24.4°. The temperature was held at from 25° to 30° throughout. The results are given in table 24.

TABLE 24.

Mgms. KF, per 100 cc.	Total liquid in cc.	Polarization after 8 days.	Polarization after 11 days.	Polarization after 13 days.
15	113	24.0	24.2	24.4
25	115	24.3	24.4	24.3
50	120	24.5	24.3	24.4

It is thus evident that the presence of milk sugar does not retard the fermentation of sucrose.

It was next undertaken to ascertain whether anything is present in milk which would interfere with the action of the fluoride. For this purpose a sample of whole milk was taken and divided into portions of fifty cc. each, to which varying amounts of fluoride were added. To each of these portions one-half cake of compressed yeast was added. The polariscopic reading of the milk before fermentation was 14.4° on the sugar scale. The readings which were obtained on five, eight, and eleven days after the beginning of the operation are given in table 25.

TABLE 25.

Mgms. KF, per 100 cc.	Original polarization.	Polarization after 5 days.	Polarization after 8 days.	Polarization after 11 days.
0	14.4	..	4.8	2.2
0	13.8	8.0	5.4	1.4
6	14.4	9.6	3.8	3.2
6	14.4	10.2	..	4.8
11	14.4	11.6	6.0	..
17	14.4	10.4	5.2	3.2
40	13.8	10.0	8.8	2.4

It will be seen that in every case there was a decided decrease in the polarized reading of the solution the fifth day and that this became more apparent the longer the operation was allowed to continue. There is evidently something present in milk which did not occur in the aqueous solution of the pure sugars which prevented in a measure the action of the fluoride in preserving lactose. It will be observed that the destruction of the lactose was less in those solutions which contained fluorides than in those which did not, and that for the first eight days the lactose was better preserved in those solutions which contained the larger amounts of fluorides than in those which contained the smaller amounts.

It was at first thought that there might be germs present in the milk, which would account to some extent for the results obtained in the experiment just cited. For this reason a sample of milk was heated to 80° and kept at that temperature for one hour, raising the temperature at the last to the boiling point for a few minutes. The sample was then allowed to cool and after four hours it was heated again as before. It was then cooled, portions of fifty cc. each were measured out and subjected to the action of yeast in the presence of fluoride as before. The original reading of the milk was 14.4°. After nine days the reading was 50.8°. This indicates that the destruction of milk sugar noted was not due to any ferments present in the milk.

It was then thought that this might be due to the presence of the casein in the milk. To ascertain this, one liter of milk was heated for about thirty minutes with ten cc. glacial acetic acid, filtered, and the filtrate polarized. Portions of this filtrate of 100 cc. each were then introduced into flasks and treated with yeast, and with amounts of potassium fluoride varying from 30 to 120 mgms. per 100 cc. of the solution. In this experiment different degrees of dilution in the filtrate were employed. The polarization of a portion of the clarified milk used in each case is given in table 26, together with the amount of fluoride used and the polarization after a number of days.

TABLE 26.

Mgms. KF, per 100 cc.	Original polarization.	Polarization after 8 days.	Polarization after 18 days.	Polarization after 20 days.
30	13.6	6.4	..	..
50	13.6	6.4	..	..
60	13.6	7.0	..	..
70	10.8	5.6	2.0	0
80	10.8	6.2	1.8	0
100	9.4	7.2	3.6	..
120	9.4	4.8	..	..

It is evident from this table that the amount of milk sugar destroyed was not by any means proportional to the amount of fluorides employed. The milk sugar seems to have been steadily consumed until after twenty days lactose could not be detected by means of the polariscope, although in this case the filtrate was so turbid that a slight error may have been made in the reading.

A sample of milk was then clarified by heating to 80° for one-half hour with a 100 cc. of a normal solution of oxalic acid to each liter. The mixture was then filtered, potassium hydroxide added to the filtrate until the reaction was almost neutral, and again filtered. This filtrate was then subjected to the action of yeast and potassium fluoride with the results given in table 27.

TABLE 27.

Mgms. KF, per 100 cc.	Original polarization.	Polarization after 5 days.	Polarization after 8 days.	Polarization after 14 days.
20	9.9	7.0	..	..
30	9.9	7.8	..	6.2
40	9.9	..	..	5.2
60	9.9	7.8	..	..
0	7.4	5.7	4.9	2.7
40	7.4	6.2	5.4	3.8

When it was desired to polarize samples of this set, they were first clarified by means of the addition of a solution of mercuric iodide in acetic acid, and a little alumina cream, made up to the mark, thoroughly mixed, and filtered. On the addition of the mercury solution a strong evolution of carbon dioxide was observed. It is probable that the potassium oxalate resulting from the neutralization of the oxalic acid was converted into potassium carbonate by the action of the yeast. This, of course, would

soon make the solution alkaline and thus interfere with the action of the fluoride.

A sample of milk was then clarified with glacial phosphoric acid in the same manner as in the preceding experiment, filtered, neutralized, and filtered again. The results as given in table 28 confirm those of the preceding experiment.

TABLE 28.

Mgms. KF, per 100 cc.	Polarization after 5 days.	Polarization after 8 days.	Polarization after 14 days.
30	..	7.1	3.6
40	7.6	7.1	4.4
60	..	8.0	4.6

We were unable to clarify milk in such a manner that the lactose would not be consumed by the organisms present in pressed yeast even in the presence of potassium fluoride. It was next attempted to add to the clarified milk something which would feed the yeast and give it such a start that it might be able to assist in holding the lactic acid ferment in check. Cane sugar seemed to be best adapted for this purpose. A sample of milk was taken and divided into portions of 100 cc. each. To each portion was added ten grams of cane sugar dissolved in fifty cc. of water, one-half cake of yeast and an amount of fluoride varying from six to forty mgms. One portion of the milk was clarified and polarized at the beginning of the operation without the addition of sucrose. The portions fermented were polarized on the eighth, eleventh, and thirteenth days. As will be seen in table 29, the original polarization of the lactose was not obtained in any case.

TABLE 29.

Sucrose, grams.	Mgms. KF, per 100 cc.	Total liquid in cc.	Original polarization lactose.	Polarization after 8 days.	Polarization after 11 days.	Polarization after 13 days.
10	6	150	14.4	10.8	10.8	8.4
10	11	150	14.4	11.8	12.0	10.6
10	17	155	14.4	14.0	12.8	11.0
10	23	155	14.4	....	13.8	12.4
10	40	165	14.4	....	....	....

Another portion of milk was sterilized by boiling, allowing to stand for four hours, and again boiling. It was then treated just as in the previous experiment with the same result. See table 30.

TABLE 30.

Sucrose, grams.	Mgms. KF, per 100 cc.	Total liquid in cc.	Original polarization lactose.	Polarization after 5 days.	Polarization after 9 days.	Polarization after 14 days.	Polarization after 20 days.
2	40	135	14.4	12.4	10.6	6.4	....
2	40	135	14.4	12.4	11.0	4.8	....
10	0	150	14.4	....	12.2	8.6	6.6
10	40	160	14.4	....	12.8	11.6	6.6

Two liters of milk were then clarified by heating to 80° for one-half hour with twenty cc. of glacial acetic acid, filtered, almost neutralized with potassium hydroxide, again filtered, and cooled to the temperature of the room. This filtrate was divided into several portions, and each portion diluted more or less with water. Each of these was again subdivided into portions of 100 cc. each, and to each of these was added a solution of ten grams of cane sugar in water, one-half cake of yeast, and an amount of fluoride varying from 0 to 100 mgms. per 100 cc. of the solution. Similar portions, of course, were polarized without the addition of sucrose. The result was still unsatisfactory, for before the fermentation of the sucrose was complete the milk sugar had begun to decompose. The results are given in table 31.

TABLE 31.

Sucrose, grams.	Mgms. KF, per 100 cc.	Total liquid in cc.	Original polarization.	Polarization after 8 days.	Polarization after 18 days.	Polarization after 20 days.
10	0	150	10.8	9.4	....	....
10	7	150	10.8	10.4	6.0	4.4
10	13	150	10.8	10.8	....	5.3
10	20	155	10.8	10.4	....	....
10	27	155	10.8	10.1	....	....
10	33	160	14.2	13.0	9.0	....
10	40	160	14.2	12.4	....	....
10	47	160	14.8	12.3	7.2	1.8
10	53	165	14.8	12.4	5.5	....
10	60	170	14.8	12.6	6.0	....
10	70	170	9.4	8.6	5.4	1.6
10	80	175	9.4	7.6	5.2	....
10	100	180	9.4	7.9	....	....

A liter of milk was then clarified with oxalic acid, filtered, neutralized, filtered, measured out in portions of 100 cc. each, ten grams of cane sugar added to each portion together with one-half cake of yeast, and a varying amount of fluoride. The

solutions were polarized after eight and fourteen days. On the eighth day the fermentation of the sucrose seemed to be complete, and no decomposition of lactose was evident. On the fourteenth day, however, the filtrates were very dark and the readings could only be taken with difficulty. There was also a very marked decrease in the amount of lactose present. The results are given in table 32.

TABLE 32.

Sucrose, grams.	Mgms. KF, per 100 cc.	Total liquid in cc.	Original polarization.	Polarization after 8 days.	Polarization after 14 days.
10	13	150	9.9	10.0	5.1
10	20	155	9.9	9.9	6.8
10	27	155	9.9	9.9	7.4
10	33	155	9.9	9.8	8.8

Two liters of milk were then heated to 80° on the steam bath, and a solution of eight grams of glacial phosphoric acid added and the whole kept at this temperature for one-half hour. At the end of this time it was filtered, neutralized, and filtered again. Portions of 100 cc. each were then measured into 200 cc. flasks, and to each was added a solution of cane sugar containing either five or ten grams of sucrose, as shown in the table, and one-half cake of compressed yeast. A portion of the filtrate was also polarized, and gave a reading of 14.4° on the sugar scale. The flasks which were set aside for fermentation were polarized, as is shown in table 33, after eight, ten, fourteen, and eighteen days.

TABLE 33.

Sucrose, grams.	Mgms. KF, per 100 cc.	Total liquid in cc.	Original polarization.	Polarization after 8 days.	Polarization after 10 days.	Polarization after 14 days.	Polarization after 18 days.
5	30	125	14.4	...	14.4	...	14.0
5	30	125	14.4	...	14.2	14.2	...
5	40	125	14.4	...	14.6	...	14.4
10	40	150	14.4	14.4	...	14.3	...
10	40	160	14.4	14.4	...	14.3	...
10	53	160	14.4	14.4	...	14.3	...

As before stated, any solution whose polariscopic reading was desired was first diluted to 100 cc. or 200 cc. In those cases in which 200 cc. was polarized the reading was multiplied by two, so that in the reading given it is always supposed that the amount of sugar present is dissolved in 100 cc. of water. It is

found that the volume of yeast was not great enough to cause any error. The fermentation of sucrose dissolved in milk whose casein had been precipitated by means of phosphoric acid was repeated several times with the same result in every instance. Whenever five to ten grams of sucrose were present at the beginning of the operation, the sucrose was completely fermented at the end of eight to ten days, while the lactose had not been affected at the end of two weeks.

The details of the application of these methods to the determination of the sugars in condensed milk are as follows:

The entire contents of the can are transferred to a porcelain dish and thoroughly mixed. A number of portions of about twenty-five grams are carefully weighed into 100 cc. flasks. Water is then added to two of the portions, and the solutions boiled to make sure that all the lactose is in solution and possesses the normal rotation. The flasks are then cooled, clarified by means of mercuric iodide dissolved in acetic acid, and alumina cream, made up to mark, filtered and polarized. Other portions of the milk are placed in a water bath and heated to the temperature of 55°. One-half cake of compressed yeast is then added to each flask and the temperature maintained at 55° for five hours. Mercury solution and alumina cream are then added, and the solution is cooled to room temperature, made up to the mark, mixed by shaking, filtered and the filtrate polarized. The amount of cane sugar present is then determined by Clerget's formula. In each case the volume of precipitated milk solids may be estimated by the double dilution method. The reducing sugar is then estimated by one of the copper reducing methods. If the amount of reducing sugar estimated as milk sugar, plus the amount of cane sugar obtained by inversion, would give a polarization equal to that obtained by the direct reading of both sugars before inversion, it is evident that no invert sugar is present, and is not necessary to test farther. If, however, the amount of reducing sugar seems to be too great, invert sugar is probably present, and the milk sugar must be determined, by another method. For this purpose 250 grams of condensed milk are dissolved in water and the solution boiled to secure the normal rotation of the milk sugar. The solution

is then allowed to cool to about 80°, a solution of $3\frac{1}{2}$ to 4 grams of glacial phosphoric acid in water is then added, and the mixture kept at this temperature for a few minutes. The mixture is then cooled to room temperature, filled up to the mark with water, thoroughly mixed by shaking and filtered.

It may be assumed that the volume of the precipitate is equal to that obtained by precipitation with mercury solution. Enough potassium hydroxide is then added to almost but not quite neutralize the free acid, and sufficient water is added to make up for the volume of the solids precipitated by the phosphoric acid. The mixture is then filtered and the filtrate is measured in portions of 100 cc. each, into 200 cc. flasks. A solution containing about twenty mgms. of potassium fluoride, and a half cake of Fleischman's compressed yeast, are then added to each flask and the mixture is then allowed to stand for ten days in a room whose temperature is 25° to 30°. The flasks are filled up to the mark, and the milk sugar is determined either by copper reduction or by means of the polariscope.

Invert Sugar.—The weight of cuprous oxide reduced by milk sugar and invert sugar, less the equivalent of the milk sugar found after fermentation, is due to invert sugar.